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Matrix metalloproteinases (MMPs) represent an important class of therapeutic targets for the treatment of diseases such as cancer. MMPs play a physiological role in the degradation of structural extra-cellular matrix (ECM) proteins and thus promote angiogenesis, a condition necessary for sustained tumor growth. Consequently, the inhibition of MMP enzymes may serve as disease-modifying agents by preventing ECM degradation and angiogenesis, and ultimately act as anti-cancer agents. In this research, we have used structure-based drug design methodologies to model selective biological inhibitors for MMPs implicated in breast cancer. Specifically, we are developing, refining, and validating computational protocols and simulations methods for docking and molecular dynamics simulations. The focus has been on validating the parameters used for molecular modeling through (1) computation of free energies of hydration, (2) flexible docking studies, and (3) evaluating Molecular Mechanics Poisson-Boltzmann Surface Area methods for computation of binding affinities. Structure-based design targeting specific MMPs will benefit from these studies by improving the accuracy of predicted binding modes and affinities of anti-breast cancer inhibitors prior to purchase or synthesis.

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## Introduction

Matrix metalloproteinases (MMPs) are a large family of zinc dependent enzymes that are involved in a number of important biological processes including embryonic development, wound repair, and tissue remodeling.[1-4] Through a highly regulated system MMPs target the structural extra-cellular matrix (ECM) for degradation and promote the formation of new blood vessels (angiogenesis). Since angiogenesis is important for the development and progression of tumor growth and cancer, specific MMP inhibitors may function as effective anti-cancer agents.[1-3] In the disease of breast cancer, which involves degradation of the extracellular matrix, inhibitors of MMPs offer a potential way to reduce the spread of primary carcinomas by slowing down metastasis and could be of significant therapeutic benefit. Enzymes found to be overexpressed in certain breast cancer tumor cell lines have included MMPs 1, 2 (gelatinase-A),[5,6] 3 (stromelysin-1),[5] as well as MMPs 7-11, 13, 14, 16.[6]

In this research we have used molecular modeling techniques to develop, validate, and refine computational protocols and simulation methods that can be used to aid in the development of anti-cancer agents targeting MMPs. We have focused on two enzymes, stromelysin-1 (MMP-3)[5] and gelatinase-A (MMP-2)[5,6] due to the fact that they have been implicated in breast cancer and crystallographic structures of protein-ligand complexes are available. Specifically, we have focused on validating the force field parameter sets used for molecular modeling calculations through (1) computation of free energies of hydration, comparing the results with published experimental hydration data, (2) flexible docking and cross docking studies of MMP inhibitors, comparing the calculated results with experimental MMP-inhibitor crystal structures, and (3) performing molecular dynamics calculations of several inhibitor/MMP complexes in order to estimate MMP activities (affinities) and selectivities. By modeling known MMP-ligand systems with reasonable accuracy, new, selective, and potent anti-breast cancer inhibitors can be proposed with greater confidence.

## Body

**Theoretical Methods.** In this study we have used calculations that incorporate a classical potential energy expression (force field) that includes Coulombic and Lennard-Jones terms to compute nonbonded interactions between the ligand and protein atoms separated by a distance $r$ (eq 1).[7]

$$E_{\text{nonbond}} = \sum_i \sum_{j>i} \left\{ \frac{q_i q_j e^2}{r_{ij}} + 4\varepsilon_{ij} \left[ \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right] \right\} \quad (1)$$

Eq 1 contains the partial atomic charges $q$ and Lennard-Jones radii and well-depths, $\sigma$ and $\varepsilon$ used to compute the pair-wise interaction energy for any given arrangement of atoms. For protein-ligand docking calculations, eq 1 is often referred to as a scoring function and can be used to rank a set of ligands on a relative basis for the electrostatic and steric complimentarity with a receptor. However, eq 1 does not include a desolvation term which, in many cases, has been shown to improve docking results[8,9] and computed free energies of binding ($\Delta G_{bind}$) in comparison with experiment.[10,11]

Including desolvation terms is expected to be especially important to accurately rank order tens of thousands of diverse ligands with a given target.

To include solvation effects for structure-based design calculations for MMPs implicated in breast cancer, as well as other chemotherapeutic targets, we are evaluating a post-docking procedure in which proposed protein-ligand complexes are processed with Molecular Mechanic Poisson-Boltzmann Surface Area (MM-PBSA) computations, as recently proposed by Srinivasen et al.[12] and reviewed by Kollman and coworkers.[10,11] In the MM-PBSA formalism the total free energy of the system (G) is computed according to eq 2.

$$G = G_{polar} + G_{nonpolar} + E_{mm} - TS \tag{2}$$

Here, a polar solvation energy term $G_{polar}$ is computed in continuum solvent using a finite Poisson-Boltzmann (PB) model and a non-polar solvation energy term $G_{nonpolar}$ is computed from a solvent-accessible surface area (SASA) calculation. Alternatively, a Generalized Born (GB) model may be used to estimate $G_{polar}$ and the method is termed MM-GBSA. The $E_{mm}$ term in eq 2 is a sum of the Coulombic, and Lennard-Jones gas-phase energies corresponding to eq 1. In eq 2, entopic effects can be included (TS term) and are typically estimated based on classical statistical formulas and normal-mode analysis of representative energy-minimized structures from a molecular dynamics trajectory. The binding free energy is then determined from eq 3.[10,11]

$$\Delta G_{bind} = G_{complex} - (G_{protein} + G_{ligand}) \tag{3}$$

It is important to note that the sum of polar (PB or GB) and nonpolar (SASA) energies in MM-PB/GBSA methods are in fact individual free energies of hydration (eq 4) if, as is commonly assumed, dielectric constants of 1 (gas-phase) and 80 (water-phase) are specified. For small neutral molecules, experimental free energies of hydration have been measured for approximately 400 compounds.[13-15]

$$\Delta G_{hyd} = G_{polar} + G_{nonpolar} \tag{4}$$

The nonpolar contributions are typically estimated as $G_{nonpolar}$ = SASA*0.00542 + 0.92, where SASA is the total molecular solvent accessible surface area of each isolated state (receptor, ligand, or complex).[10,11] In this work, atom-based SASAs were also computed and fit to the difference in experimental $\Delta G_{hyd}$ and PB polar energies using multiple linear regression (eq 5) in order to improve agreement with experiment.

$$\Delta G_{hyd} - G_{polar} = G_{nonpolar} = \sum_i c_i SASA_i \tag{5}$$

Although reasonable correlation with experimental free energies of binding have been reported for several systems, despite the approximations made in MM-PBSA, more studies should be performed in order to further validate and test the method. In particular, larger and more diverse data sets should be considered and application of MM-

PBSA and MM-GBSA methods to post-score docking results should be evaluated. Given that the accuracy of docking and MM-PB/GBSA calculation results are primarily influenced by the quality of the force field parameters used to evaluate the energy of the system, parameter set validation is critical.

**Free Energies of Hydration.** To gauge the accuracy of including desolvation penalties from continuum desolvation calculations in our docking and binding energy calculations of breast cancer targets, we have computed free energies of hydration for comparison with experiment.[13-15] We have expanded upon the work detailed in our previous Annual Summary Report (June 13, 2002) by increasing the size of the neutral data set from 265 to 410 molecules as well as including additional charges models. A total of six charge models, Gasteiger/MOE[16], MFF94/MOE[16], AM1-BCC/AMBER7,[17] ChelpG HF/6-31G*,[18] and Merz-Kollman HF/6-31G*[18] have been evaluated. PB calculations were performed with the DelPhi[19] program, GB calculations were performed with AMBER7[20], ChelpG and MK calculations were performed with Gaussian98,[18] and SASA calculation were performed with both MOLSURF[20], and DMS[21] programs.

**Docking and Cross-docking Studies.** Docking calculations were initiated from three MMP-ligand co-complexes (pdb entries 1USN,[22] 2USN,[22] and 3USN[23]) to determine if the calculations could regenerate known binding modes of the ligands to the stromelysin receptors. Cross-docking calculations were also pursued to determine if each inhibitor could be placed into the different experimental stromelysin binding pockets independent of which inhibitor was associated with which experimental complex. For the docking calculations, standard protein and ligand Lennard-Jones nonbonded parameters were assigned from the vdw.defn file distributed with DOCK 4.0.1.[24] Charge assignment for each receptor was performed with the SYBYL[16] implementation of the AMBER force-field.[25] Ligand charges were derived using the AM1-BCC[17] method as implemented in AMBER7.[20] The catalytic zinc ion used for the MMPs was from Stote et al., zinc charge $q = +2\ e^-$, $\sigma = 1.7$ Å, $\varepsilon = 0.67$ kcal/mol.[26] The Stote model was shown to yield the best overall results out of eight zinc parameter sets tested for docking ligands to thermolysin[27] when compared with experiment. More recently, MM/PBSA[28] calculations have used the Stote zinc model to rank binding energies for six known carboxylate ligands of stomelysin-1 (MMP-3) in reasonable agreement with experiment.[29]

We have developed a new protocol for docking that expands upon our efforts that detailed in the last Annual Summary Report (June 13, 2002). For each ligand docked, 50 low energy binding modes are saved, ranked for affinity with the receptor, and then clustered into groups according to heavy atom root mean square deviation (rmsd) similarity. The top-scoring cluster member (the cluster-head) for the top five clusters are then subjected to post-processing using the MM-PBSA procedure as described above. We find that a cutoff similarity of 2.00 Å yields reasonable cluster sizes with cluster members occupying the same regions of space. The goal of this new procedure is to retain 5 (more if desired) low scoring and diverse binding modes from the docking calculations. All docking calculations in the present study were performed with DOCK 4.0.1.[24]

**Molecular Dynamics Simulations.** Molecular dynamics (MD) calculations have been performed to determine if theoretical binding affinities computed using MM-GBSA methods follow experimental trends for the selectivity between stromelysin (MMP-3) and gelatinase-A (MMP-2) for two 5-substituted-1,3,4-thiadozole-2-thiones. Using

crystallographic structures of PNU-142372 bound to stromelysin (pdb entry 1USN)[22] and gelatinase-A complexed with a hydroxamate inhibitor (pdb entry 1QIB)[30] as starting coordinates, MD simulations were initiated in continuum solvent using AMBER7[20] with a GB solvation model. The ligands were assigned AM1-BCC charges and the GAFF[20] force field using the antechamber package within AMBER7. Standard PARM94[20] parameters were assigned to the stromelysin and gelatinase-A receptors. Simulations employed a 1 femtosecond time step for 40010 steps corresponding to 40.01 picoseconds of GB molecular dynamics. The final desired temperature of 298 was obtained by requesting a heating cycle from 0 to 298 kelvin over the course of the first 5000 molecular dynamics steps with temperature regulation maintained via coupling to an external heat bath using the Berendsen scheme.[31] Average structural and energetic quantities used to the estimate binding affinities for the MMP inhibitors and selectivities between stromelysin and gelatinase-A receptors were computed using 200 snapshots from the last 20.01 picoseconds of the MD trajectory.

**Key Research Accomplishments**

**Hydration Free Energies**

- GB and PB $G_{polar}$ calculations were performed using six different charge models for a data set consisting of 410 neutral organic molecules with experimental free energies of hydration. This expands upon our previous work that considered 265 neutral compounds. Excellent correlation was observed between the GB and PB calculations results as shown in Figure 1 for the RESP charges molecules (correlation coefficient $r^2 = 0.97$). Calculations using other charges models also yielded good agreement between the two theoretical methods. Such good correlations support the use of the much faster GB method as implemented in AMBER7 for estimating the $G_{polar}$ term used to compute the $\Delta G_{hyd}$ component for protein-ligand studies targeting breast proteins.

- PB $G_{polar}$ calculation results obtained using the six different charge models have been plotted against experimental free energies of hydration (Figures 2-3). AM1-BCC, ChelpG HF/6-31G*, Merz-Kollman HF/6-31G*, RESP, and MFF94 charge models all yield reasonable linear correlation with experimental $\Delta G_{hyd}$ as shown in Figures 2-3 and listed in Table 1 (column A). This expands upon our previous work that considered only two charge models. Five partial charge models significantly outperformed the Gasteiger model (Figure 3, Table 1 column A) previously used in our laboratory for structure-based design. Given this fact, if accurate solvation energies are important, Gasteiger charges would not be recommended in calculations targeting breast cancer enzymes.

- $G_{nonpolar}$ energies were computed from molecule-based SASA using standard conversion constants ($G_{nonpolar} = 0.00542 + 0.92$) and plotted against the difference in $\Delta G_{hyd}$ (experimental) and $G_{polar}$ energies. No correlation was observed (Figure 4, Table 1 column B) which is surprising given that molecule-based SASA $G_{nonpolar}$ energies are routinely added to $G_{polar}$ in order to estimate $\Delta G_{hyd}$. An alternative procedure to estimate $G_{nonpolar}$ procedure was pursued which included computation of atom-based SASAs and using multiple linear

regression fitting methods to determine an optimal coefficient for each SASA type (Table 2). With this procedure, atom-based $G_{nonpolar}$ energies were computed that yield much better agreement with experiment as shown in Figure 5 for the RESP charged molecules (green triangles). Also shown in Figure 5 are the nearly constant $G_{nonpolar}$ energies obtained from molecule-based SASA contributions using the standard constants (black squares, red circles). Figure 6 shows the improved agreement between the computed and experimental free energies of hydration obtained using the new atom-based SASAs with the RESP charge set (red circles) vs. molecule-based SASAs (black squares). For completeness, Table 2 shows all optimized coefficients from the different atom-based SASA fittings using the six different charged datasets.

- Correlations have been computed between $G_{nonpolar}$, obtained using the RESP fit SASA constants, and the difference in $\Delta G_{hyd}$ (experimental) and PB $G_{polar}$ energies (Table 1, column C) for each of the six different charge models. These calculations are used to determine if RESP fit SASA constants are transferable to calculations that use other charge schemes. In Table 1, significant improvement between the compute and experimental free energies of hydration is observed using atom-based SASAs (RESP fit constants) for ChelpG, RESP, and MK calculations (Table 1 columns D vs. E). Atom-based SASA calculations (RESP fit constants) for AM1-BCC, Gasteiger, and MFF94 calculations for $\Delta G_{hyd}$ yield diminished agreement with experiment. For the AM1-BCC calculations the decrease in $r^2$ from 0.80 to 0.68 (Table 1, columns D vs. E) is surprising given AM1-BCC was parameterized to mimic RESP charges. One possible explanation comes from the fact that empirical adjustment was made in the original AM1-BCC parameterization in order to yield better experimental free energies of hydration better than RESP. Therefore, while RESP and AM1-BCC methods yield similar charge distributions for many molecules, some compounds yield substantially different $G_{polar}$ energies implying that different $G_{nonpolar}$ contributions would be necessary to obtain agreement with experiment requiring different SASA constants.

**Docking and Cross-docking Studies.**

- Flexible docking experiments have been performed for three 5-substituted-1,3,4-thiadozole-2-thiones to stromelysin (Figure 7) for comparison with previously reported structural data. Figure 8 shows the best re-scored result (green) compared with experiment (red) that was obtained using our new docking, clustering, and MM-PBSA protocols. Binding modes in agreement with experiment were obtained for compounds PNU-142372 with 1USN (rmsd = 0.5) and PNU-141803 with 2USN (rmsd = 2.53) but differ substantially for PNU-107859 (rmsd = 5.51). Poor results in the latter case may be due to the fact that coordinates used in the docking came from NMR as opposed to x-ray; docking procedures have typically been calibrated to reproduce x-ray structures. The close agreement with experiment for the X-ray derived structures (Figure 8) support the use of the Stote zinc model, AM1-BCC ligand charges, and new docking protocols targeting proteins implicated in breast cancer.

- Flexible cross-docking calculations have been performed in which each of the three inhibitors was docked into the three different stromelysin receptors 1USN, 2USN, 3USN (Table 3, Figure 9). The calculations successfully placed ligands PNU-142373 (red) and PNU-141803 (green) into 1USN in the same orientation as observed crystallographically (Figure 9 bottom left) although PNU-107859 (blue) shows deviation. For docking to 2USN, again the calculations for PNU-142373 (red) and PNU-141803 (green) show reasonable agreement with experiment and PNU-107859 (blue) is not placed correctly. In general poor results were obtained for cross-docking to the NMR derived structure 3USN.

- Previous work (Annual Summary Report May 2001) required the zinc binding group (ZBG) for each ligand be specified as the "anchor" prior to docking, in order to provide reasonable agreement with respect to experimental binding modes. In the present study thiadiazole ZBGs were not manually defined; ligand placement was determined during the docking calculations from energetics alone. For breast cancer research projects that aim to computationally screen hundreds of thousands of diverse ligands, manual definition of ZBGs would be problematic.

**Molecular Dynamics Simulations and MM-GBSA Calculations**

- Continuum solvent GB molecular dynamics simulations of two different 5-substituted-1,3,4-thiadozole-2-thiones (Table 4) inhibitors have been performed with both stromelysin and gelatinase-A receptors. Figure 10 shows the close structural homology between the two enzymes. The MD trajectories were post-processed with MM-GBSA from the final 20.02 picoseconds using snapshots saved every 0.10 picoseconds. The MM-GBSA calculations were able to correctly predict that both compounds bind better to stromelysin than gelatinase-A, and the relative affinities between the two compounds with either target alone agree with experiment. Experimentally the fluoro compound (PNU-142372) shows greater activity by ca –2 kcal/mol with stromelysin and –1.4 kcal/mol with gelatinase-A (Table 4). Notably, the MM-GBSA calculations parallel experiment and predict greater activity for PNU-142372 by ca –4 kcal/mol with stromelysin and –3.5 kcal/mol with gelatinase-A in good agreement with experiment (Table 4). For breast cancer targets in particular (MMPs), the ability to discriminate between such highly homologous enzymes is an important tool that can be used towards the development of selective anti- cancer agents.

**Reportable Outcomes**

- The previous PI (Samual Toba, Ph. D) developed a unified combinatorial library docking methodology for use in the design of multiple libraries against multiple targets.[32]

**Design, Docking, and Evaluation of Multiple Libraries Against Multiple Targets**

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**ABSTRACT:** We present a general approach to the design, docking, and virtual screening of multiple combinatorial libraries against a family of proteins. The method consists of three main stages: docking the scaffold, selecting the best substituents at each site of diversity, and comparing the resultant molecules within and between the libraries. The core "divide-and-conquer" algorithm for side-chain selection, developed from an earlier version (Sun et al., J Comp Aided Mol Design 1998;12:597-604), provides a way to explore large lists of substituents with linear rather than combinatorial time dependence. We have applied our method to three combinatorial libraries and three serine proteases: trypsin, chymotrypsin, and elastase. We show that the scaffold docking procedure, in conjunction with a novel vector-based orientation filter, reproduces crystallographic binding modes. In addition, the free-energy-based scoring procedure (Zou et al., J Am Chem Soc 1999;121:8033-8043) is able to reproduce experimental binding data for P1 mutants of macromolecular protease inhibitors. Finally, we show that our method discriminates between a peptide library and virtual libraries built on benzodiazepine and tetrahydroisoquinolinone scaffolds. Implications of the docking results for library design are explored.

- March 18th, 2001 the current PI Robert C. Rizzo, Ph. D. assumed responsibility for grant DAMD17-00-1-0192 (Modification P00001) from the previous PI Samual Toba, Ph. D.

- Research poster presentation 1 (abstract below):
  The Sixteenth Meeting of Groups Studying the Structures of Aids Related Systems and Their Application to Targeted Drug Design, Sponsored by the National Institute of General Medical Science and National Institutes of Health, June 19 - June 21, 2002, Lister Hill Auditorium, National Institutes of Health, Bethesda, Maryland.

- Research poster presentation 2 (abstract below):
  Gordon Research Conference in Computational Chemistry, June 30 - July 5, 2002, Colby-Sawyer College, New London, NH.

- Abstract for research poster presentations 1 and 2:
**Database Mining for Compounds to Inhibit HIVgp41 Mediated Viral-host Cell Membrane Fusion**

Robert C. Rizzo, Miriam Gochin, and Irwin D. Kuntz*

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**ABSTRACT:** Viral-host cell membrane fusion events during HIV infection are mediated by viral envelope proteins gp120 and gp41. X-ray studies of the core domain of HIVgp41 have revealed a hairpin structure (fusion-active) in which three outer C-helices loop and wrap around three inner N-helices. Peptides based on both N and C helices have been reported which target the pre-hairpin structure (intermediate) and disrupt the formation of the fusion-active state thereby inhibiting HIV viral-host cell membrane fusion. We are using the DOCK suite of programs to screen compounds from the Available Chemicals Directory and National Cancer Institute for compatibility with the highly conserved hydrophobic pocket formed at the interface of the gp41 N-helices. Favorably docked compounds will be tested for the ability to bind to the gp41 N-helices using an NMR-based assay and for anti-HIV activity using a cell-based assay.

- Research poster to be presented 3 (abstract below):
The Seventeenth Meeting of Groups Studying the Structures of Aids Related Systems and Their Application to Targeted Drug Design, Sponsored by the National Institute of General Medical Science and National Institutes of Health, June 18 - June 20, 2003, Lister Hill Auditorium, National Institutes of Health, Bethesda, Maryland.

- Research poster submitted 4 (abstract below):
The 226th American Chemical Society National Meeting, September 7 - September 11, 2003, New York, NY

- Abstract for research posters 3 and 4:
**Targeting the disruption of HIVgp41 mediated cell membrane fusion: Docking and MM-GB/PBSA studies**

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**ABSTRACT:** Cell membrane fusion events during HIV infection are mediated by viral envelope proteins gp120 and gp41. Crystallographic studies of the core domain of HIVgp41 have revealed a hairpin structure (fusion-active) in which

three outer C-helices loop and wrap around three inner N-helices. Peptides based on the outer C-helices have been reported which target a proposed pre-hairpin intermediate and disrupt formation of the fusion-active state thereby inhibiting cell membrane fusion. We are using the DOCK suite of programs to virtually screen compounds from the National Cancer Institute, targeting a highly conserved hydrophobic pocket formed at the interface of the gp41 N-helices. For each docked compound, the fifty best scoring complexes are ranked, clustered, and re-ranked using MM-PBSA and MM-GBSA calculations to include estimates of desolvation. The most favorable re-ranked compounds will be tested for the ability to bind to a gp41 pre-hairpin model using fluorescence and NMR-based assays.

- A protocol for computing and fitting atom-based SASA as an alternative to using molecule-based constants was established for free energy of hydration calculations.

- A protocol for post-scoring results from DOCK calculations was devised in which low energy binding modes are ranked for affinity with the receptor, clustered into groups according to heavy atom root mean square deviation similarity, and post-processed using the MM-PBSA methods.

- AM1-BCC charges have been incorporated into the Available Chemical Directory (ACD)[33] and National Cancer Institute (NCI) [34] used in our laboratory for molecular docking, data-mining, molecular dynamics, and MMPB/GBSA calculations.

**Conclusions**

Consistent with the original Statement of Work (SOW) objectives we are continuing to develop, refine, and validate computational protocols and simulations methods used to model proteins that have been implicated in breast cancer. Our studies have included protocol and parameter development towards modeling hydration effects know to be important in protein-ligand binding. Docking, cross docking, and molecular dynamics calculations were then pursued for breast cancer targets stromelysin and gelatinase. In each case, emphasis was placed on validating the theoretical methods, protocols, and parameters used to model each system by comparing theoretical results to experimental data to insure that the computational predictions are efficient and accurate.

Results from GB and PB calculations reveal that the two different theoretical methods are comparable as shown in Figure 1 for the RESP charged neutral molecules. The excellent correlation provides reasonable assurance that the less computationally demanding GB method can be used in place of PB methods to estimate polar contributions to the free energy of hydration. For structure-based design studies targeting breast cancer proteins GB methods would be preferred given that PB calculations would be time prohibitive for screening large virtual databases.

To gauge the contribution and accuracy of computed $G_{polar}$ terms to the calculated free energies of hydration, experimental $\Delta G_{hyd}$ were plotted for the 410 molecules against PB $G_{polar}$ results obtained using six different charge models (Figures 2-3). Reasonable

linear correlations with experiment were obtained using five of the charge models AM1-BCC/AMBER7, ChelpG HF/6-31G*, Merz-Kollman HF/6-31G*charges, and MFF94 (Figures 2-3), however, low correlation with experiment was observed using the Gasteiger charges historically used in our laboratory (Figure 3).

The importance of including a nonpolar term based on SASAs, to the total computed free energies of hydration, was investigated by plotting $G_{nonpolar}$ against the difference between the experimental $\Delta G_{hyd}$ and $G_{polar}$. Assuming that $G_{polar}$ calculations are exact, nonpolar contributions plotted against $\Delta G_{hyd}$ minus $G_{polar}$ should be linear. In Figure 4, no correlation is apparent using the standard constants, $G_{nonpolar}$ = SASA*0.00542 + 0.92, only a constant energetic contribution of 2-3 kcal/mol is obtained. These standard constants were originally obtained by fitting SASA results to $\Delta G_{hyd}$ for a series of linear alkanes in which the $G_{polar}$ term was reasonably assumed to be negligible (i.e. zero). However, for more diverse data sets, such as presented here, this assumption is not valid and $G_{polar}$ contributions contribute significantly to $\Delta G_{hyd}$ as shown in Figure 2.

Given the lack of correlation observed between experiment and theory using molecule-based SASAs, a procedure was developed in which atom-based SASA were computed and atom-based SASA coefficients were obtained from multiple linear regression fitting using eq 5 (Table 2). Breakdown of the atomic SASAs was element-based with the exception of hydrogen atoms that were classified according to their bonded partner with hydrogen connected to carbon (HC), sulphur (HS), nitrogen (HN), or oxygen (HO) as unique (Table 2). Although, improved agreement with experiment was observed using atom-based SASA constants as shown in Figure 6 for the test molecules set up with RESP charges, it would be desirable to have one set of atom-based SASA constants that could be used with all charge models. In Table 1, good transferability was achieved between ChelpG, RESP, and MK methods but transferability of RESP derived atom-based SASA coefficients for AM1-BCC, Gasteiger, and MFF94 charge calculations led to diminished agreement with experiment.

Flexible docking and cross-docking experiments for 5-substituted-1, 3, 4-thiadozole-2-thione ligands to stromelysin yielded binding modes for the ligands in reasonable agreement with the crystallographic complexes 1USN[22] and 2USN[22] (Figures 7-9, Table 3). These calculations provide support for our flexible docking, clustering, and rescoring protocols, and help to validate our choice of the Stote nonbonded zinc model[26] AM1-BCC[17] charge models for docking to MMPs. Calculations initiated using the NMR derived complex 3USN[23] showed significant deviation from experiment and in this case the use of an NMR derived receptor (held rigid during the docking) may be problematic.

Molecular dynamics MM-PBSA calculations have yielded calculated free energies of binding consistent with available experimental data for the selectivity between stromelysin and gelatinase-A and were able to reproduce the relative difference in binding between two inhibitors that only differ by a fluorinated aromatic ring (Table 4). Such good correlation with experiment highlights the utility of using computational methods to study not only relative affinities of MMP inhibitors implicated in breast cancer but selectivities between different MMPs as well.

Developing potent and selective chemotherapeutics with minimal side effects remains an important goal in breast cancer research. The zinc dependant MMPs have

become an attractive drug target due to their role in the development and progression of tumor growth and cancer through degradation of the extracellular matrix. With the recent proliferation of available crystallographic structures of MMPs including stromelysin-1 (MMP-3) and gelatinase-A (MMP-2) implicated in breast cancer, structure-based drug discovery and development tools can be applied towards the design of potent and selective MMPs inhibitors. It is anticipated that the application of computational protocols, methods, and parameters developed here will ultimately improve the ability of theoretical methods to yield meaningful results by improving the accuracy of predicted binding modes and affinities of potential chemotherapeutics prior to purchase or synthesis.

**Figure 1.**

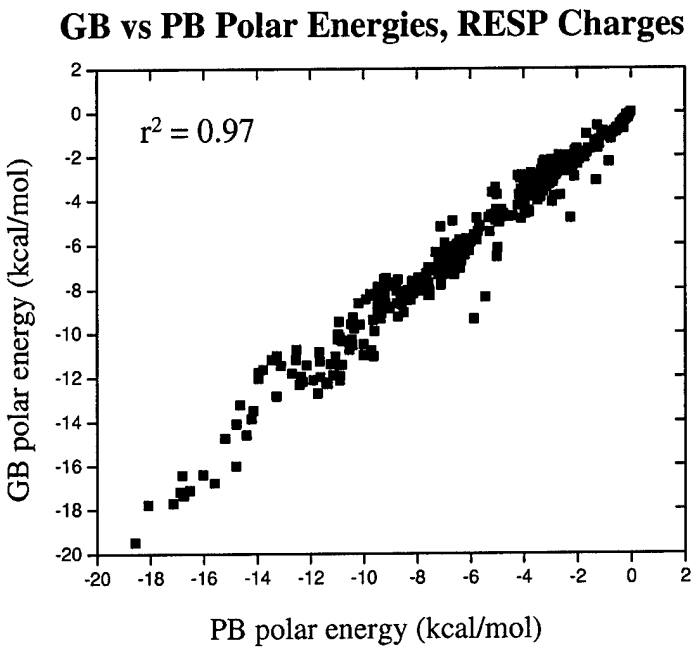



**Figure 2.**

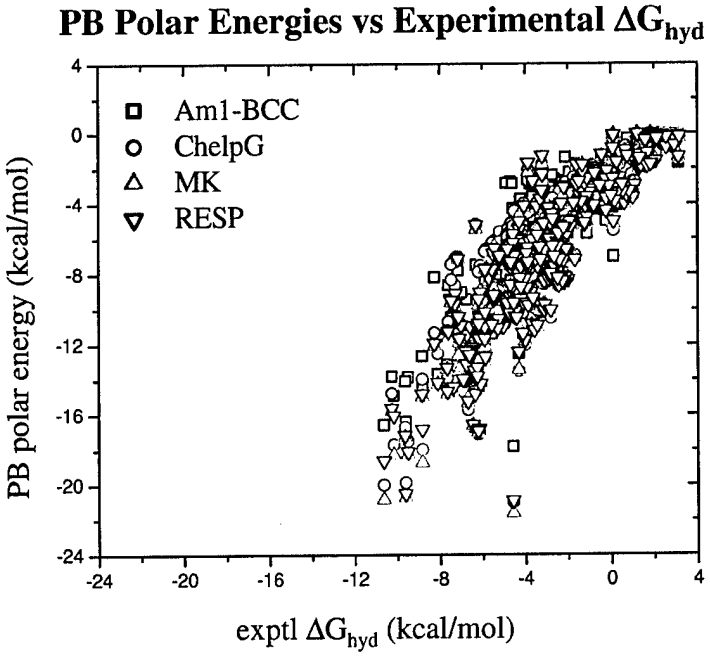


**Figure 3.**

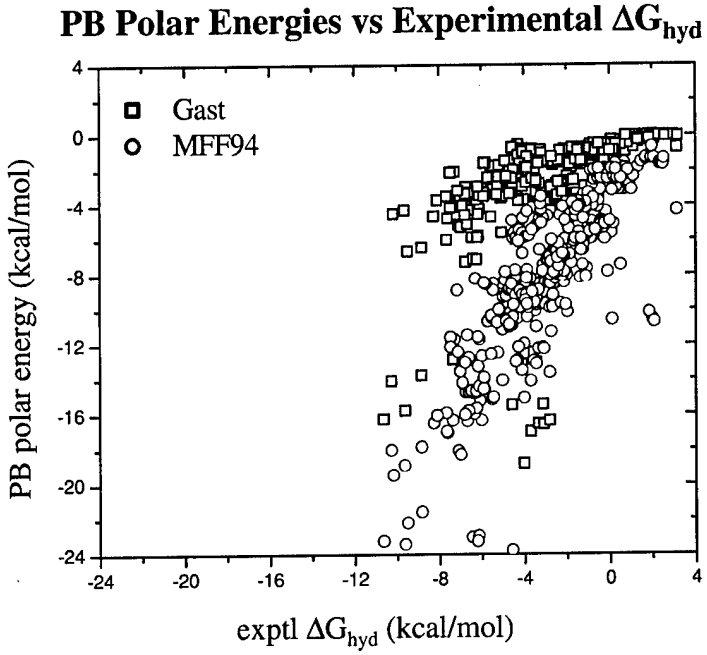



**Figure 4.**

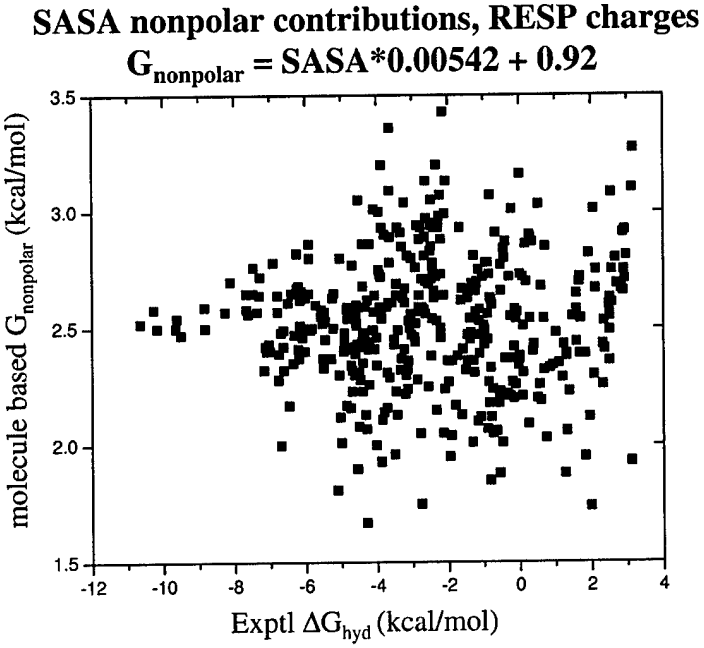


**Figure 5.**

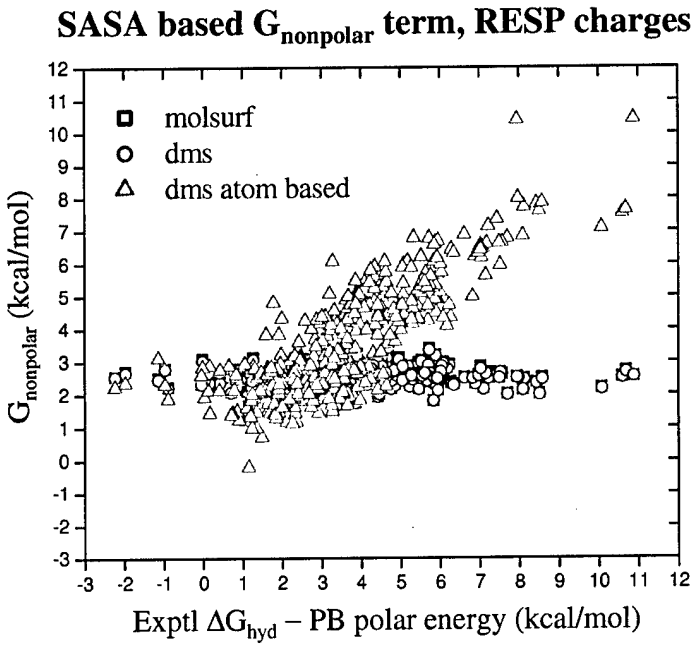



**Figure 6.**

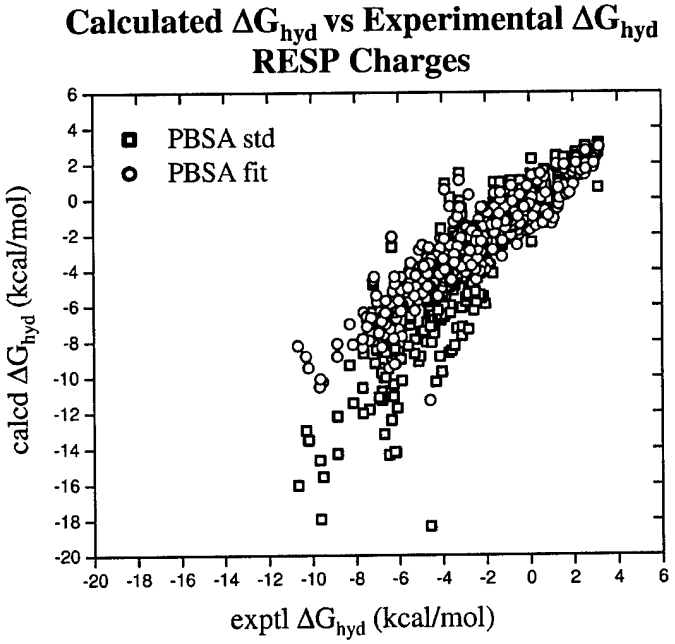


Figure 7.

**Stromelysin molecular surface showing inhibitor PNU-142372 in red, (pdb entry 1USN)**

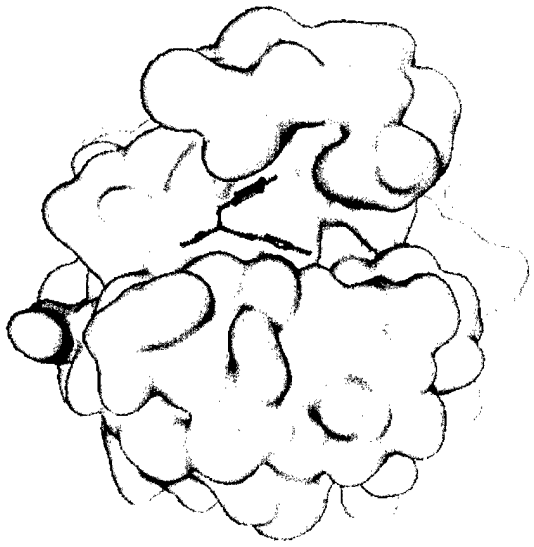


Figure 8.

**Flexible docking results: experimental (red) vs predicted (green) binding modes for thiadiazole inhibitors with stromelysin**

| PNU-142372 | PNU-141803 | PNU-107859 |
|---|---|---|
| rmsd = 0.50 | rmsd = 2.53 | rmsd = 5.51 |
| pdb entry 1USN | pdb entry 2USN | pdb entry 3USN |
| X-ray | X-ray | NMR |

Figure 9.

**Cross-docking results for PNU-142372 (red), PNU-141803 (green), and PNU-107859 (blue)**

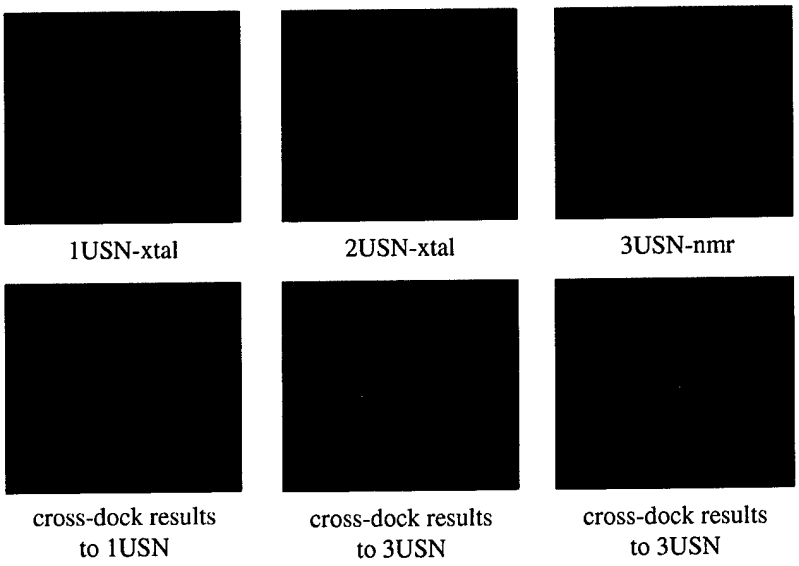



Figure 10.

**Structural similarity between stromelysin (red) and gelatinase-A (green)**

**Table 1.** Correlation coefficients for $\Delta G_{hyd}$ (experiment) vs. PB $G_{polar}$, $G_{nonpolar}$ standard, $G_{nonpolar}$ RESP fit Ci's, $\Delta G_{hyd}$ standard (calculated), and $\Delta G_{hyd}$ RESP fit Ci's (calculated).

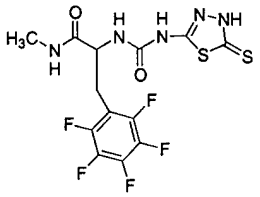
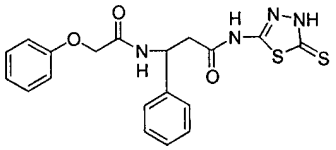
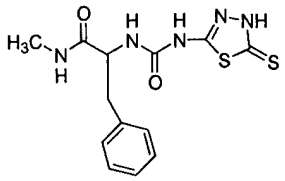
| Charge Model | $G_{polar}$ PB A | $G_{nonpolar}$ standard[a] B | $G_{nonpolar}$ RESP fit Ci's[b] C | $\Delta G_{hyd}$ standard (calcd) D | $\Delta G_{hyd}$ RESP fit Ci's (calcd) E |
|---|---|---|---|---|---|
| **ChelpG** | 0.74 | 0.00 | 0.68 | 0.74 | 0.81 |
| **RESP** | 0.78 | 0.00 | 0.68 | 0.79 | 0.84 |
| **MK** | 0.79 | 0.00 | 0.72 | 0.79 | 0.86 |
| **AM1-BCC** | 0.79 | 0.00 | 0.30 | 0.80 | 0.68 |
| **Gasteiger** | 0.31 | 0.00 | 0.022 | 0.31 | 0.076 |
| **MFF94** | 0.74 | 0.00 | 0.54 | 0.74 | 0.71 |

[a] $G_{nonpolar}$ standard = (SASA*0.00542+0.92). [b] $G_{nonpolar}$ RESP fit Ci's = ($SASA_i$*$C_i$).

**Table 2.** Regression coefficients from multiple linear regression methods obtained by fitting atom based SASAs to the difference in experimental free energies of hydration and PB $G_{polar}$ energies ($\Delta G_{hyd} - G_{polar}$) for the six different charge models tested.

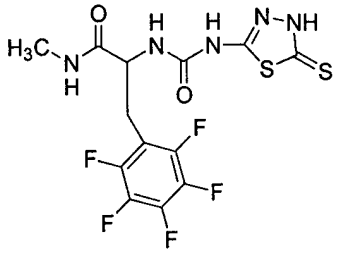
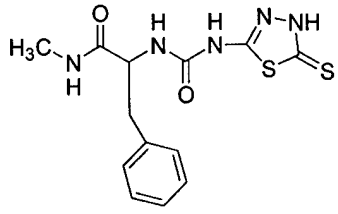
| Type | ChelpG | RESP | MK | AM1-BCC | Gasteiger | MFF94 |
|---|---|---|---|---|---|---|
| HC | 0.00680 | 0.00719 | 0.00699 | 0.00451 | 0.00229 | 0.00689 |
| HO | 0.163 | 0.177 | 0.192 | 0.287 | –0.434 | 0.08224 |
| HN | –0.01299 | –0.00613 | –0.00808 | 0.00231 | –0.04156 | –0.02005 |
| C | –0.00539 | 0.01287 | 0.01604 | 0.02858 | –0.02369 | 0.03487 |
| N | 0.03475 | 0.02275 | 0.02467 | 0.00548 | –0.04035 | 0.08498 |
| O | 0.06997 | 0.05996 | 0.06352 | 0.01576 | 0.05060 | 0.08630 |
| F | 0.02319 | 0.02042 | 0.02193 | 0.02825 | 0.02225 | 0.03915 |
| S | –0.00486 | –0.00745 | 0.00205 | 0.00256 | –0.02485 | 0.01103 |
| Cl | 0.00678 | 0.00515 | 0.00557 | 0.00245 | 0.00175 | 0.01724 |
| Br | 0.01374 | 0.01783 | 0.02051 | 0.00288 | –0.00671 | 0.01648 |
| I | 0.00715 | 0.01064 | 0.01309 | 0.03956 | –0.00883 | –0.00477 |
| HS | — | — | — | — | — | — |
| P | — | — | — | — | — | — |
| | $r^2 = 0.75$ | $r^2 = 0.68$ | $r^2 = 0.73$ | $r^2 = 0.54$ | $r^2 = 0.44$ | $r^2 = 0.72$ |

**Table 3.** Cross-docking results for thiadiazole inhibitors to stromelysin (MMP-3). RMSD (Å) values are computed between docked and experimental ligand positions.

| Name | Structure | RMSD (Å) PDB number | | | Exptl Activity |
|---|---|---|---|---|---|
| | | 1USN | 2USN | 3USN | Ki (uM) |
| **PNU-142372 1USN** |  | 0.50 | 3.75 | 6.99 | 0.018[a] |
| **PNU-141803 2USN** |  | 2.05 | 2.53 | 3.81 | 0.31[a] |
| **PNU-107859 3USN** |  | 5.99 | 5.72 | 5.51 | 0.710[b] |

[a]Reference[22]. [b]Reference[23].

**Table 4.** Computed MM-GBSA binding energies ($\Delta G_{bind}$) from GB-based molecular dynamics simulations for thiadiazole inhibitors with stromelysin (MMP-3) and gelatinase-A (MMP-2). Estimated experimental binding energies $\Delta G_{bind} \approx RT \ln$ (activity) in kcal/mol.

| | | **stromylesin-1** | | **gelatinase-A** | |
|---|---|---|---|---|---|
| **Name** | **Structure** | **exptl[a]** | **calcd** | **exptl[a]** | **calcd** |
| **PNU-142372 (fluro)** |  | –10.57 | –35.40 | –7.53 | –25.17 |
| **PNU-107859 (hydro)** |  | –8.39 | –31.37 | –6.15 | –21.65 |

[a]Reference[35].

**Acronyms and Definitions**

| | |
|---|---|
| AM1-BCC | Austin Model 1 Bond Charge Correction, atomic partial charges model. |
| AMBER | Assisted Model Building with Energy Refinement, a suite of program that uses molecular mechanics to model proteins and ligands, also may refer to the force field parameters used by the various programs. |
| ChelpG | ChelpG atomic partial charges model. |
| DMS | A program used to compute both molecular and atomic SASA, solvent accessible surface areas. |
| DOCK | A program used to predict binding modes (geometric arrangements) between potential ligands with a receptor. The process is called docking. |
| $G_{polar}$ | Polar component of free energy of hydration estimated from PB or GB calculations. |
| $G_{nonpolar}$ | Nonpolar component of free energy of hydration estimated from SASA calculations. |
| $\Delta G_{hyd}$ | Free energy of hydration, energy required for a molecule to go from gas-phase to water. |
| $\Delta G_{bind}$ | Free energy of binding, energy of association between a ligand and receptor. |
| GAFF | General Amber Force Field, generalized empirical force field appropriate for ligands and organics molecules. |
| GB | Generalized Born, refers to a computational procedure to estimate the energy to transfer a molecule from a region of low dielectric to high dielectric (solvation energy). |
| HF/6-31G* | Hartree Fock quantum mechanical method incorporating a 6-31G* basis set, used compute electrostatic potentials that yield partial atomic charges. |
| Merz-Kollman | Merz-Kollman atomic partial charge model. |
| MFF94, | Merck Force Field 1994, atomic partial charge model. |

| | |
|---|---|
| MM | Molecular Mechanics, a method used to compute structures and energies of molecules, generally proteins and ligands, that incorporates empirical parameters (force field) fit to agree with experimental data. |
| MMPs | Matrix metalloproteinases, a family of proteins implicated in breast cancer. |
| MM-GBSA | Molecular Mechanics Generalized Born Surface Area, a procedure used to estimate the binding affinity for a ligand with its receptor. |
| MM-PBSA | Molecular Mechanics Poisson-Boltzmann Surface Area, a procedure used to estimate the binding affinity for a ligand with its receptor. |
| MOLSURF | A program used to compute molecular SASA, solvent accessible surface areas. |
| PB | Poisson-Boltzmann, refers to a computational procedure to estimate the energy to transfer a molecule from a region of low dielectric to high dielectric (solvation energy). |
| SA or SASA | Solvent Accessible Surface Area, the molecular or atomic surface area that could be available to interact with solvent (usually water). |

**Statement of Work (SOW) Generalized Summary**

**Task1:** Develop an accurate zinc ion model molecular mechanics force field representation in matrix metalloproteinases for use in molecular docking (months 1-12).

- A series of testing and refinements using several zinc models and force fields on known crystal structures of matrix metalloproteinase enzyme-ligand complexes (MMP-1 and MMP-3) was performed. It was found that a small modification to the non-bonded model of Stote et al. makes it most suitable for use in docking and can well reproduce the crystallographic zinc coordination state (Annual Summary Report May 2001).

- Calculations revealed that the use of zinc binding group (ZBG) "anchor-based" helps to correctly identify the electrostatic complementarity between the zinc and the ZBG functional groups such as hydroxamate or carboxylic acid (Annual Summary Report May 2001).

- Recent calculations for thiadiazole inhibitors using AM1-BCC charges indicate that manual definition of ZBGs may not be necessary given that good ligand placement was determined during the docking calculations from energetics alone. This is significant for projects that aim to computationally screen thousands or millions of diverse ligands to breast cancer targets; manual definition of ZBGs can be problematic.

- Additional force field parameter studies were undertaken to gauge the accuracy of including desolvation penalties from continuum desolvation calculations in our docking and binding energy calculations of breast cancer targets. We have expanded upon the work detailed in the previous Annual Summary Report (June 13, 2002) by increasing the size of the neutral data set from 265 to 410 molecules as well as including additional charges models. In addition, a protocol for computing and fitting atom-based SASA as an alternative to using molecule-based constants was established for free energy of binding calculations.

**Task2:** Use the parameters developed (Task 1) and the techniques of molecular docking to identify potent and selective non-peptidyl inhibitors of stromelysin-1 and MT1-MMP (months 13-36).

- Docking studies were undertaken to include a series of known MMP-1 and MMP-3 inhibitors and commonly occurring non-cytotoxic drugs as negative controls. In both cases, we were able to selectively distinguish the known inhibitors from the other drugs based on the DOCK free energy score. Docking results for approximately 300 antineoplastic agents show a preference for many pyrimidine-like molecules for MMPs (Annual Summary Report May 2001).

- A new protocol for post-scoring results from DOCK calculations was devised in which low energy binding modes are ranked for affinity with the receptor,

clustered into groups according to heavy atom root mean square deviation similarity, and post-processed using the MM-PBSA methods.

- Flexible cross-docking calculations using the new protocol were performed in which each of the three inhibitors was docked into the three different stromelysin receptors. The calculations successfully placed two out of three ligands into 1USN and 2USN in reasonable orientations compared to those observed crystallographically. In general, poorer results were obtained for cross-docking using an NMR derived structure 3USN.

**Task 3:** Develop structure-based combinatorial library and apply molecular docking methodologies to obtain potent and selective inhibitors for stromelysin-1 and MT1-MMP (months 13-36).

- Induced fit mechanisms that lead to potency and selectivity for MMP inhibitors have been explored using docking, comparative modeling, and molecular dynamics of the protein TACE and identified the regions of high mobility within the TACE active site. Comparison with crystallographic thermal factors between the native and complexed structure of Adamalysin (closest protein to TACE; native crystal structure unavailable) have identified the "bottleneck" region as being the loop and helix around the S1' pocket. MD simulations confirm this fact and show ~ 4 A of mobility for the same region as in S1'. Our test with mutations to Alanine for selected "blocking residues", coupled with the "soft docking" approach have shown good promise as an ad-hoc approach to estimate induced fit (Annual Summary Report May 2001).

- Selectivity and affinity between breast cancer targets stromelysin-1 (MMP-3) and gelatinase-A (MMP-2) using molecular dynamics (MD) calculations have been performed and show that theoretical binding affinities computed using MM-GBSA methods follow the experimental trends for the selectivity for 5-substituted-1,3,4-thiadozole-2-thiones. These calculations highlight the utility of using computational methods to predict not only relative affinities of inhibitors targeting MMP but selectivity between different proteins as well. Specific matrix metalloproteinase inhibitors could reduce side effects associated with breast cancer treatment by targeting individual enzymes implicated in the disease.

**Personnel Receiving Pay from this Research Effort**

- Robert C. Rizzo, Ph.D. (Current PI, March 18 2002 - May 30 2003)
- Samual Toba, Ph.D. (Former PI, May 15 2000 - May 01 2001)

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